Sperm deoxyribonucleic acid integrity decreases with age and exhibits a rapid decline beyond the age of 35: a retrospective evaluation of 3446 semen samples

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INTRODUCTION

In recent decades, birth rates have substantially increased for men older than 30 years because of advanced age of marriage, rising life expectancy at birth, modern societal norms, and accessibility to assisted reproductive technology (ART). Advanced paternal age has been associated with a decline in conventional semen parameters (volume, concentration, motility, as well as, reduced fertility, increased risk of miscarriage, structural chromosomal aberrations and complex epigenetic disorders.

Multiple studies have underlined the significance of sperm DNA integrity test, as a part of routine semen analysis, a key component which affects functional competence of the sperm. Furthermore, several etiological factors such us obesity, smoking, genital tract infection, chemotherapy, varicocele, irradiation, leukocytospermia have been associated in the impairment of sperm DNA integrity.

Sperm with high DNA fragmentation may rise the possibility of transmitting the genetic aberrations to the conceptus and may affect the embryo and post natal development. Such transfer of aberrant sperm genome may result in increased rates of miscarriage or birth of offspring with major or minor congenital malformation.

The effect of paternal age on semen quality and integrity is controversial. Several studies with different measurement techniques (TUNEL, SCD, SCGE, SCSA) in the literature have recommended testing sperm DFI in infertile men with advanced age (≥40 years) as it may provide prognostic information for couple attempting natural and assisted reproduction.

STUDY QUESTION

Does advancing paternal age correlate with sperm DNA fragmentation index (DFI) and is there a cut-off age beyond which sperm DFI increases significantly?

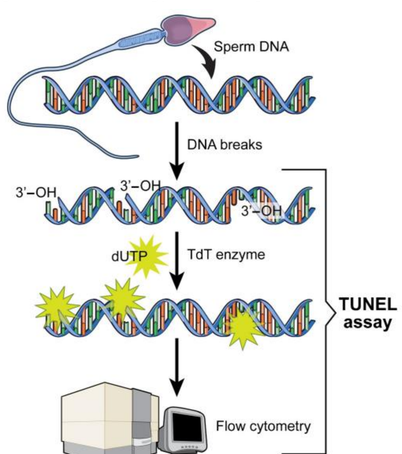
METHODS

Study design: This is a retrospective study of 3446 semen samples from patients under investigation for infertility between April 2016 and January 2022. Semen samples were obtained after 2-3 days of sexual abstinence. Patients were stratified into seven groups based on their age: patients ≤ 29 years (n=127; 3.7%), 30-35 years (n=868; 25.2%), 36-39 years (n=863; 25.0%), 40-45 years (n=1017, 29.5%), 46-49 years (n=321; 9.3%), 50-55 years (n=179, 5.2%) and ≥ 56 years (n=71, 2.1%).

Sperm DNA fragmentation analysis: Conventional semen parameters were assessed according to the WHO criteria and DFI was evaluated by TUNEL assay using the APODirect Kit run on BDAccuriC6 flow cytometer. A cut-off of 16.9% for DFI was applied to classify normal vs fragmented samples.

Statistics: Pearson's r was used for correlation analysis between sperm concentration, DFI and paternal age. DFI results for each stratified patient group were evaluated by one-way ANOVA, followed by Tukey pos-hoc multiple comparison test. Results are presented as the mean±standard error and a P-value of <0.05 was considered statistically significant.

Figure 1. Diagram of the DNA staining procedure using the TUNEL method

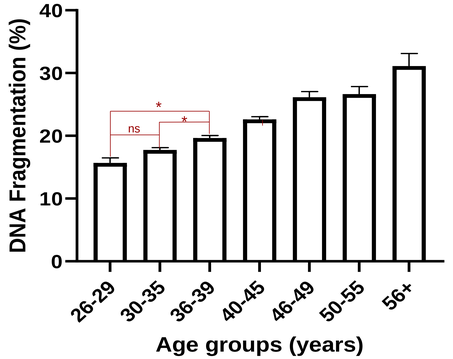


RESULTS

Table 1. Descriptive statistics of 3446 men attending OVO fertility clinic and sperm DNA integrity

Age group	26-29	30-35	36-39	40-45	46-49	50-55	56+
Number of values	127	868	863	1017	321	179	71
Minimum	1,9	0,4	0,8	1,0	3,0	2,9	0,9
Maximum	38,4	70,6	93,7	98,9	118,4	88,4	78,5
Range	36,5	70,2	92,9	97,9	115,4	85,5	77,6
Mean DFI	15,68	17,74	19,65	22,61	26,15	26,66	31,12
SD	9,07	11,55	12,05	13,7	16,21	15,77	16,94
SEM	0,81	0,39	0,41	0,43	0,91	1,18	2,01
Lower 95% CI of mean	14,09	16,97	18,84	21,76	24,37	24,34	27,11
Upper 95% CI of mean	17,28	18,51	20,45	23,45	27,93	28,99	35,13

Figure 2. Association between age and sperm DNA fragmentation in patients who follow up at the OVO fertility clinic



In this cohort of men with a mean age of 39.5 years ± 0.1 (range 23-76 years), sperm DNA fragmentation (21.1% ± 0.2) was positively correlated with age (r=0.23, p<0.001). In contrast, the correlation between sperm concentration and age was not significant (r=0.03, p=0.07). Mean %DFI level in the 26-29 and 30-35 age groups were not significantly different (p=0.65). However, mean %DFI level in the 36-39 age group was significantly higher than in the 26-29 and 30-35 age groups (p=0.02 and p=0.03, respectively). Mean %DFI level in the older age groups (40-45, 46-49, 50-55 and ≥ 56 years) were all significantly higher than in the 26-29 or 30-35 age groups (p<0.001). Using a %DFI threshold level of 16.9%, 46.0% of patients <36, 52.2% of men aged 36-39, 60.2% of men aged 40-45, 67.3% of men aged 46-49, 72.6% of men aged 50-55 and 74.7% of men aged ≥ 56 years had an elevated DFI.

CONCLUSIONS

Our results underline the relationship between paternal age and sperm DFI and demonstrate a significant increase in sperm DNA fragmentation in men over the age of 35 years. The data suggest that we may want to reconsider the age cut-off we traditionally use to define advanced paternal age.

Limitations: This is a retrospective analysis that did not account for confounding variables (e.g., clinical diagnosis, gonadotoxin exposure, febrile illness) that may affect conventional sperm parameters and DFI.

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